BACTERIOLOGICAL SURVEYS OF THE ST. MARY'S RIVER (1973-74), SERPENT RIVER AND SPANISH RIVER (1975)

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Ministry of the Environment

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BACTERIOLOGICAL WATER QUALITY OF THE ST. MARY'S RIVER IN 1973 AND 1974

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MICROBIOLOGY SECTION
LABORATORY SERVICES BRANCH
MINISTRY OF THE ENVIRONMENT

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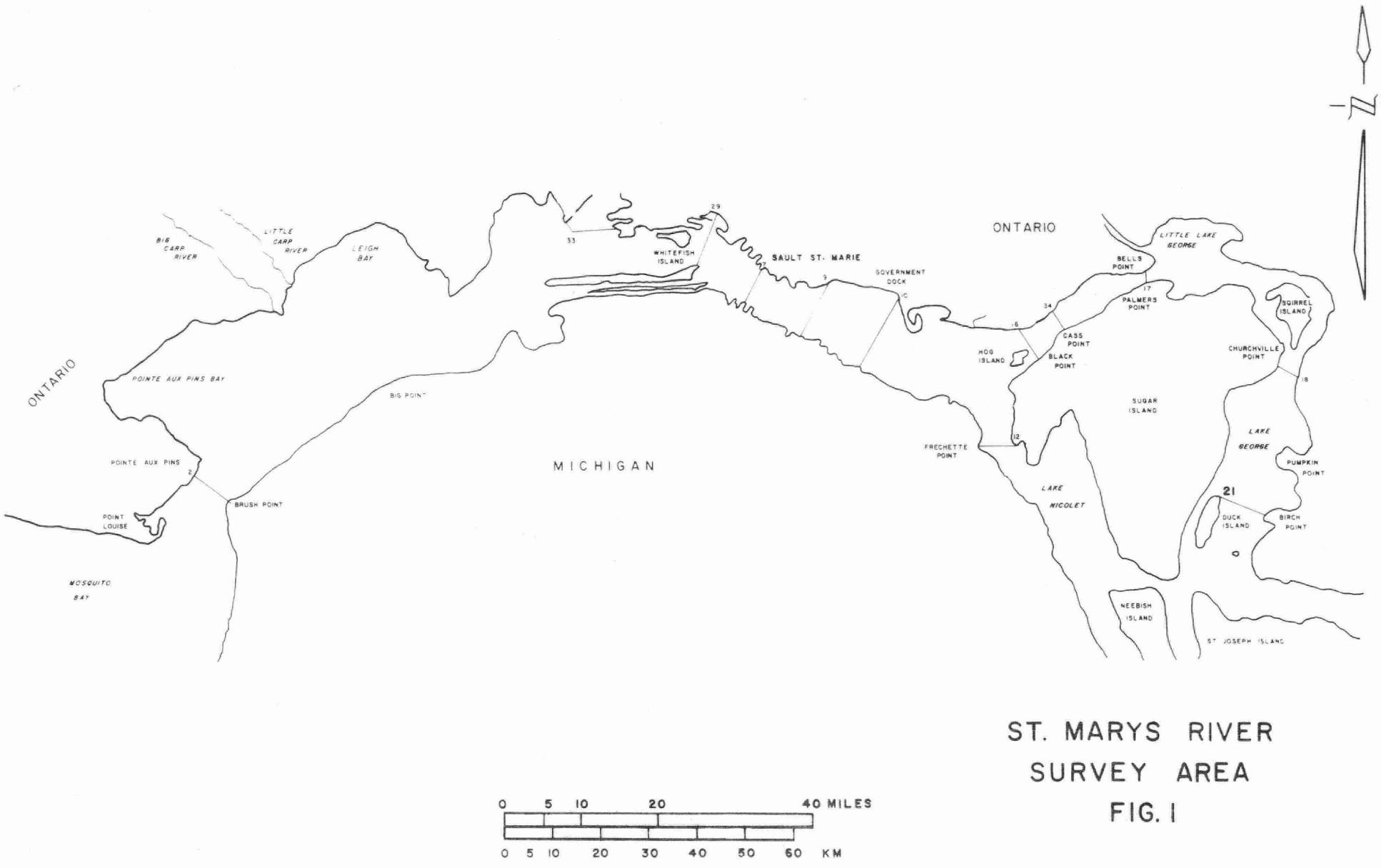
Abstract

Bacterial populations of the St. Mary's River were determined in 1973 and 1974 as part of the M.O.E. - IJC continuing program to help assess overall water quality and problem areas of the river, and to determine whether significant bacterial population changes occurred during the two years. Bacterial densities, especially below the locks, were consistently higher towards the Canadian shore. The spring and summer 1974 surveys generally revealed higher concentrations than the corresponding 1973 surveys. Bacterial contamination was only evident in the south branch of the river leading to Lake Nicolet during July 1973.

Area Description

St. Mary's River (Fig.1), the interconnecting waterway of Lakes Superior and Huron, is important because of the river's multiple use potential. Its uses encompass municipal, industrial, navigational, and recreational waters. Because its flow can be controlled by a Compensating Works Dam and because of the presence of locks, commercial and pleasure craft are fully able to exploit the river. Sugar Island, one of the main islands, diverts the river below the locks into two channels: Lake Nicolet (the west channel) and Lake George (the international waters). Neebish Island further subdivides the river from Lake Nicolet into the west and middle channels while St. Joseph Island subdivides the flow from Lake George into the middle and east channels.

Situated along the Sault Ste. Marie Ontario shoreline are numerous industries. The Algoma Steel Corporation, the Abitibi Paper Company, the Mannesman Tube Company, and the Dominion Tar and Chemical Company constitute main sources of employment in the area.



ST. MARYS RIVER SURVEY AREA FIG. 1

Objectives

1) 1973

"To assess the existing water quality in terms of its compliance with Ministry criteria and IJC objectives, the causes of any violations of the permissable or desired levels, the materials input to the river from Lake Superior and the materials output to Lake Huron and the occurrence of transboundary movement of pollution. To assist in the recommendation of abatement measures as necessary for the correction or prevention of water use conflicts."[1]

2) 1974

"To determine the downstream dispersion (lateral and longitudinal) of oil, phenolics, ammonia and cyanides coming from Algoma Steel Corporation. To appraise the need for additional remedial measures."

Analytical Methods

Samples were taken at selected ranges along the river (Table 1). These ranges (where applicable) extend from the American to the Canadian shoreline. All samples were collected 1 metre below the surface. In 1973, a modified "piggy-back" sampler and sterile 237 ml evacuated rubber syringes were used. Samples were stored on ice until they arrived at the mobile laboratory within 12 hours of sampling at which time they were transferred aseptically to sterilized 250 ml polycarbonate bottles. In 1974, all samples were collected in 175 ml sterile glass bottles. Analysis for total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) using the membrane filtration technique (MF) as described in Standard Methods[3] were performed using m-Endo agar Les (Difco) for TC and MacConkey membrane broth (Oxoid), along with an incubation period of 18 hours, for FC determinations.

Table 1

ST. MARY'S RIVER RANGES SAMPLES IN 1973 AND 1974

1 9 7 3		1 9 7 4	
Range	Distance (ft) from U.S. Shore	Range	Distance (ft) from U.S. Shore
2	500, 1000, 1300, 1600, 1900, 2200	29	100, 400, 600, 700, 800, 875
33	500, 900, 950, 975	7	100, 1500, 2300, 2400, 2450
29	100, 400, 600, 700, 800, 875	9	200, 1500, 2200, 2500, 2600, 2700
7	100, 1500, 2300, 2400, 2450	10	2100, 3000, 3500, 4000, 4200
10	100, 500, 1500, 2100, 3000, 3500 4000, 4200	16	200, 600, 900
17	100, 500, 900	34	200, 700, 1200, 1700, 2100, 2200
12	100, 500, 900	17	100, 500, 900
		18	200, 600, 1000, 1200
		12	100, 500, 900

In 1974 heterotrophic bacteria (HB) concentrations were determined by the spot plate technique.

In June and August 1974 surveys, Pseudomonas aeruginosa (P.aer.) were determined using the MF technique and mPA medium.[5]

Statistical Methods

Water Quality cannot be assessed accurately from a single sample because of changing environmental conditions. Therefore a large number of samples were taken to satisfy these conditions. Statistical methods were used to summarize the results concisely and to reduce biased interpretation of the data. An analysis of viariance programme (ANOVA) was used to summarize the data.

In this programme the calculated F ratio must be less than the critical F ratio (0.05 level) in order that the stations comprise a statistically similar group. If the F was significant, then those stations in the river with significantly different geometric means (G.M.) were deleted from the overall group to yield a group with similar means. Stations comprised a group provided that they were not separated by any geographic barrier, that the variances of all the stations were similar (Bartlett's X^2 Test of Homogeneity) and that the data were normally distributed.

Using the ANOVA program again, calculations were done on the deleted stations. This process was repeated until all possible groups were formed. The Student-t test (Using Log G.M. and S.E.) was then used to compare overlapping homogeneous ares for the surveys within any given year and between the two years.

Criteria

The Ministry of the Environment (MOE 1974) criteria states "where ingestion is probable, recreational waters can be considered impaired with the coliform, fecal coliform and/or enterococcus geometric mean density exceeds 1000, 100, and or/ 20 per 100 ml respectively in a series of at least ten samples per month, including samples collected during weekend periods".[6]

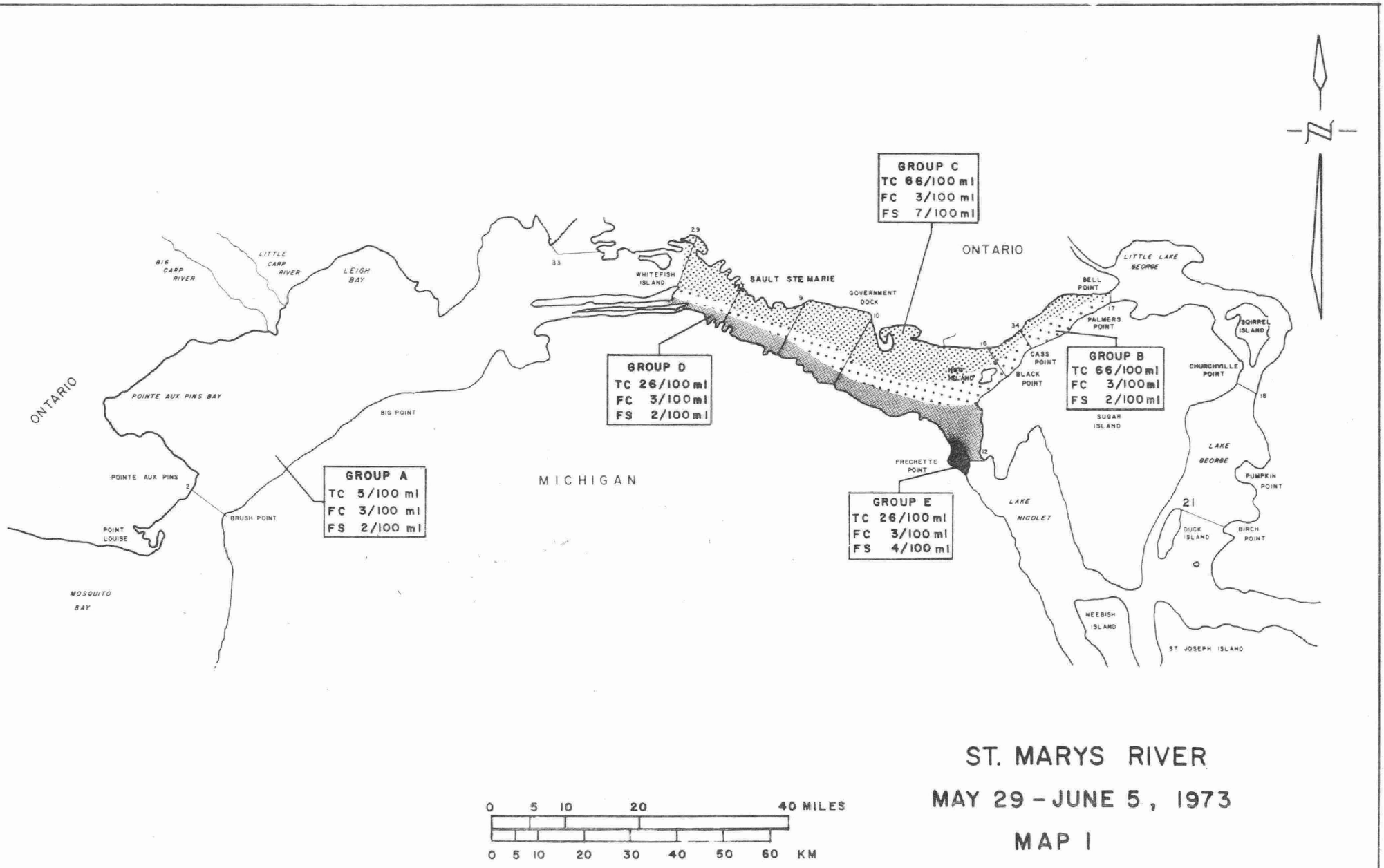
"Where the ratio of fecal coliforms to fecal streptococci (calculated from geometric means) exceeds 4.0, the source of bacterial contamination is likely to be human in origin. A ratio of less than 0.7 indicates non human source of bacterial contamination".[7]

This ratio must be used at or in the immediate vicinity of the source. In addition it does not impart information of the safety of the water as animals can harbour human pathogens.

The maximum permissible criteria for public raw water supplies with full treatment are 5000 TC, 500 FC, 50 FS/100 ml and 100,000 total heterotrophic bacteria/100 ml.

Results & Discussion 1973:

In May (Map 1), the entire upstream area of the river (above the locks) had very low TC concentrations of 5/100 ml (Grp.A). Downstream from the locks on the Canadian side of the river, an increased TC level of 66/100 ml occurred (Grp.B) whereas on the American side, the TC level was 26/100 ml (Grp.D). FC densities were homogeneous throughout the river at 3 FC/100 ml. The channel on the Canadian side below the locks (Grp.C) had higher FS than FC densities, suggesting a non-human source of contamination in this area.



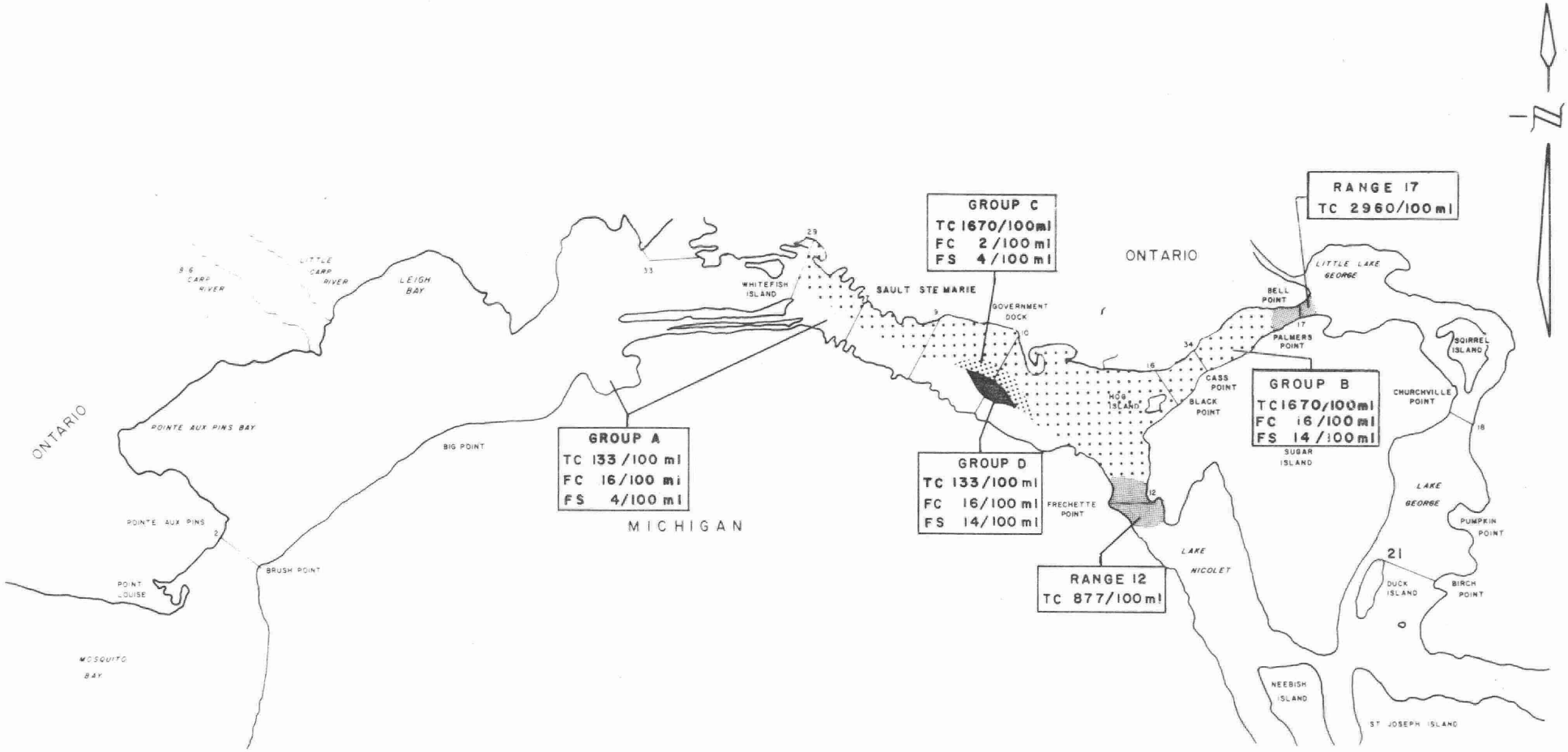
ST. MARYS RIVER

MAY 29 – JUNE 5, 1973

MAP I

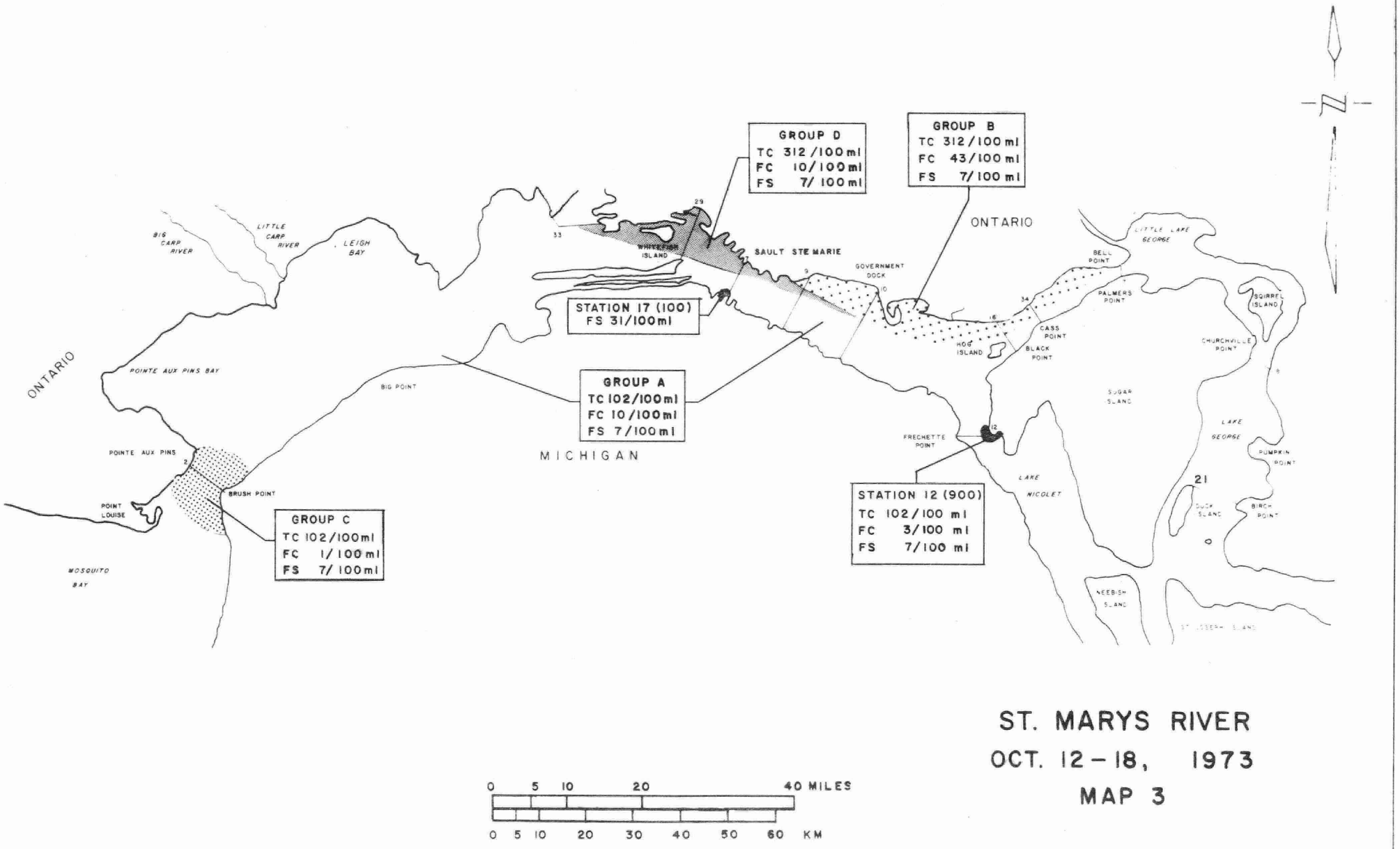
In July (Map 2), all upstream stations and most of the downstream stations towards the American side of the river had 133 TC and 16 FC/100 ml (Grp.A) while the downstream stations on the Canadian side and part of the channel leading to Lake Nicolet had 1670 TC and 16 FC/100 ml (Grp.B). The area at the end of the northern channel had a high TC density of 2960 and 22 FC/100 ml (Range 17). The upstream stations had greater FC than FS concentrations (Grp.A) while the same trend occurred for the downstream stations on the Canadian side and part of the channel leading to Lake Nicolet (Grp.B). This suggested that contamination was mainly of human origin. The cause of the higher TC bacterial densities at the last range before Lake George (Range 17) might be due to a combination of nutrient enrichment and discharges from the Sewage Treatment Plant, located on the Canadian Shore between ranges 16 and 34.

In October (Map 3) the upstream stations and those closest to the American shoreline below the locks had 102 TC/100 ml (Grp.A) while the Canadian downstream side had a higher TC level of 312/100 (Grp. D). These two areas had a FC level of 10/100 ml. Both the upstream and downstream areas had higher FC than FS concentrations, suggesting contamination of human origin was predominating downstream, possibly as a result of the influence of the STP.



ST. MARYS RIVER
JULY 25 - JULY 29, 1973
MAP 2

5A



ST. MARYS RIVER
OCT. 12 – 18, 1973
MAP 3

Summary 1973

Bacterial populations for most areas sampled above the locks in both American and Canadian waters formed a homogeneous group for the May, July, and October surveys.

Densities in May were generally low, however, the increased levels that occured during the July and October surveys remained well within the MOE Recreational Use Criteria.

Below the locks, bacterial levels in Canadian waters were generally higher than those of the American waters during the three surveys.

In May, all areas sampled below the locks in both American and Canadian waters had higher bacterial levels than the upstream stations but still well within the MOE Recreational Criteria.

In July, most of the river had higher bacterial levels than in May. The MOE TC Recreational Use Criteria was exceeded during the July survey below the locks close to the heavily industrialized Canadian shoreline and the area sround the northern arm leading to Lake George.

In October bacterial concentrations at most stations decreased from the July survey with the exception of FC concentrations in the Canadian waters downstream from the locks. The higher FC might be due to the storm sewer effluent near the Government wharf and the STP.

Flow patterns are such that a greater volume of water passes through Lake Nicolet than the channel around the northern arm leading to Lake George. Thus concentrations of contaminants along the Canadian shoreline would be higher than those of the American shoreline not only because of the more heavily industrialized Canadian shoreline but because of a slower flow of water along the Canadian side.

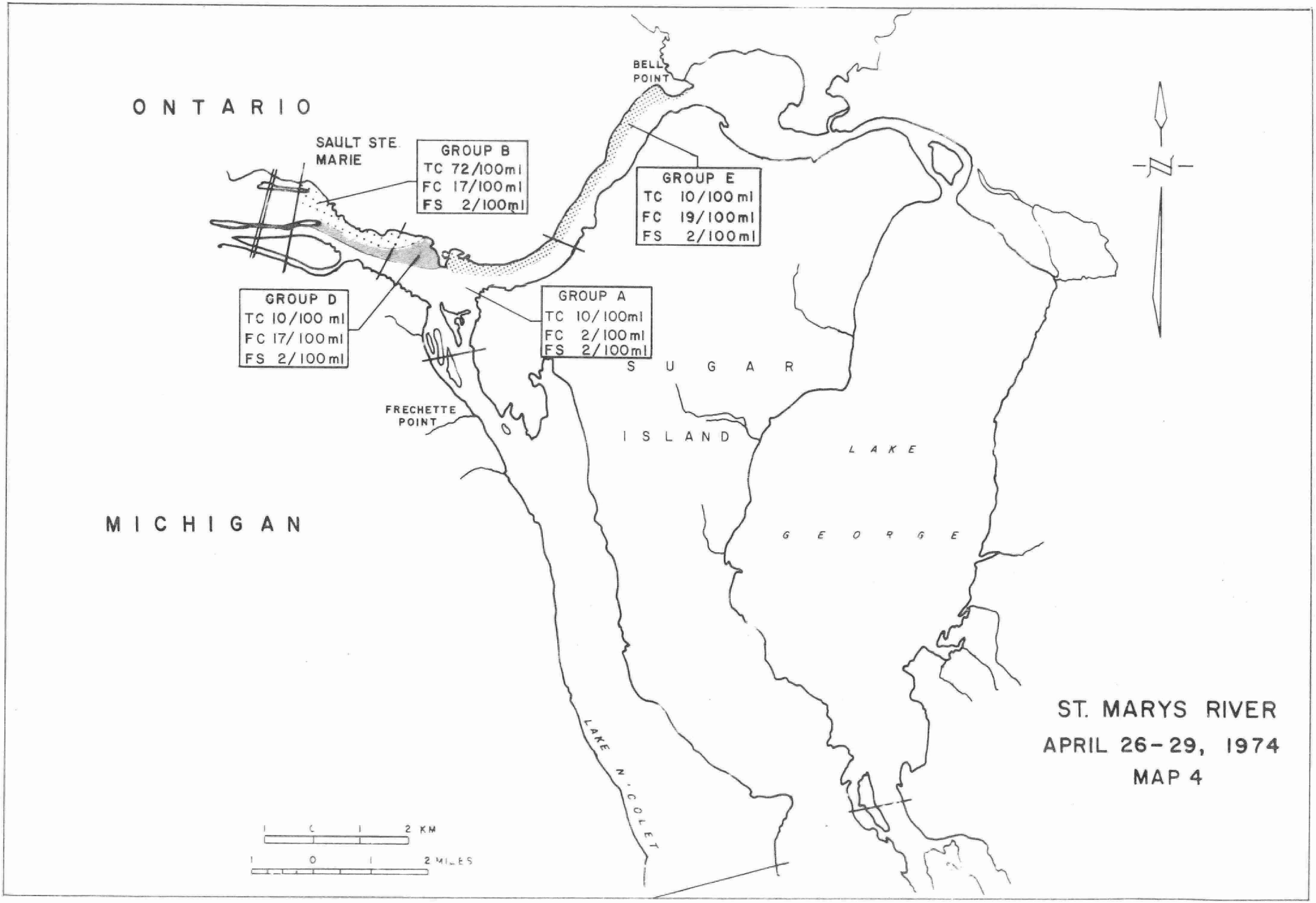
RESULTS AND DISCUSSION 1974.

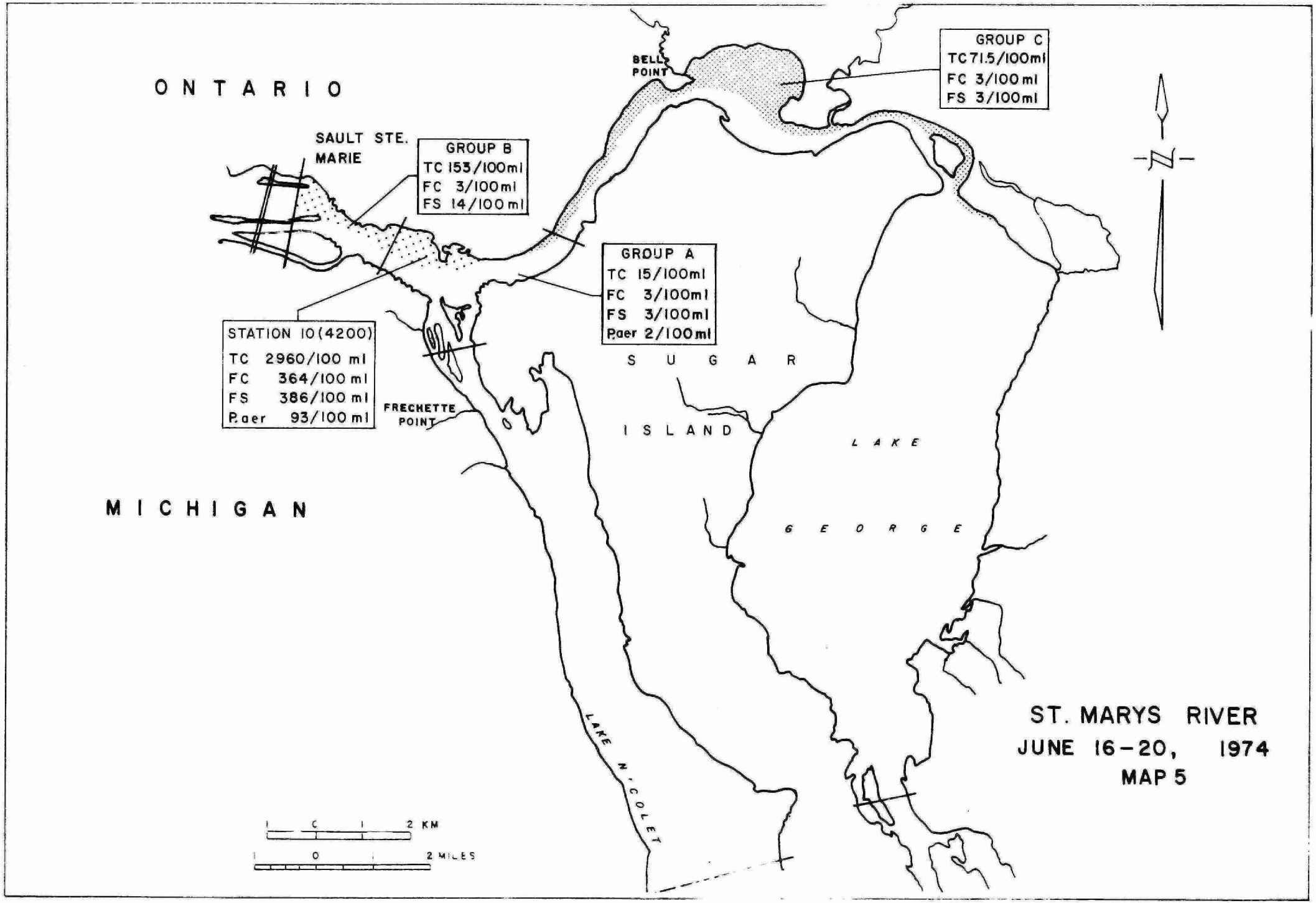
The April Survey (Map 4) revealed the majority of river ranges to have bacterial densities of 10 TC, 2 FC and 2 FS/100 ml (Grp.A). The Canadian side beginning downstream from the locks to Black Point had slightly higher levels of 72 TC, 17 FC and 2 FS/100 ml (Grp. B). The areas between Black and Palmer's had greater FC than FS concentrations once again indicating contamination of human origin from the heavily populated Canadian side and the Sewage Treatment Plant.

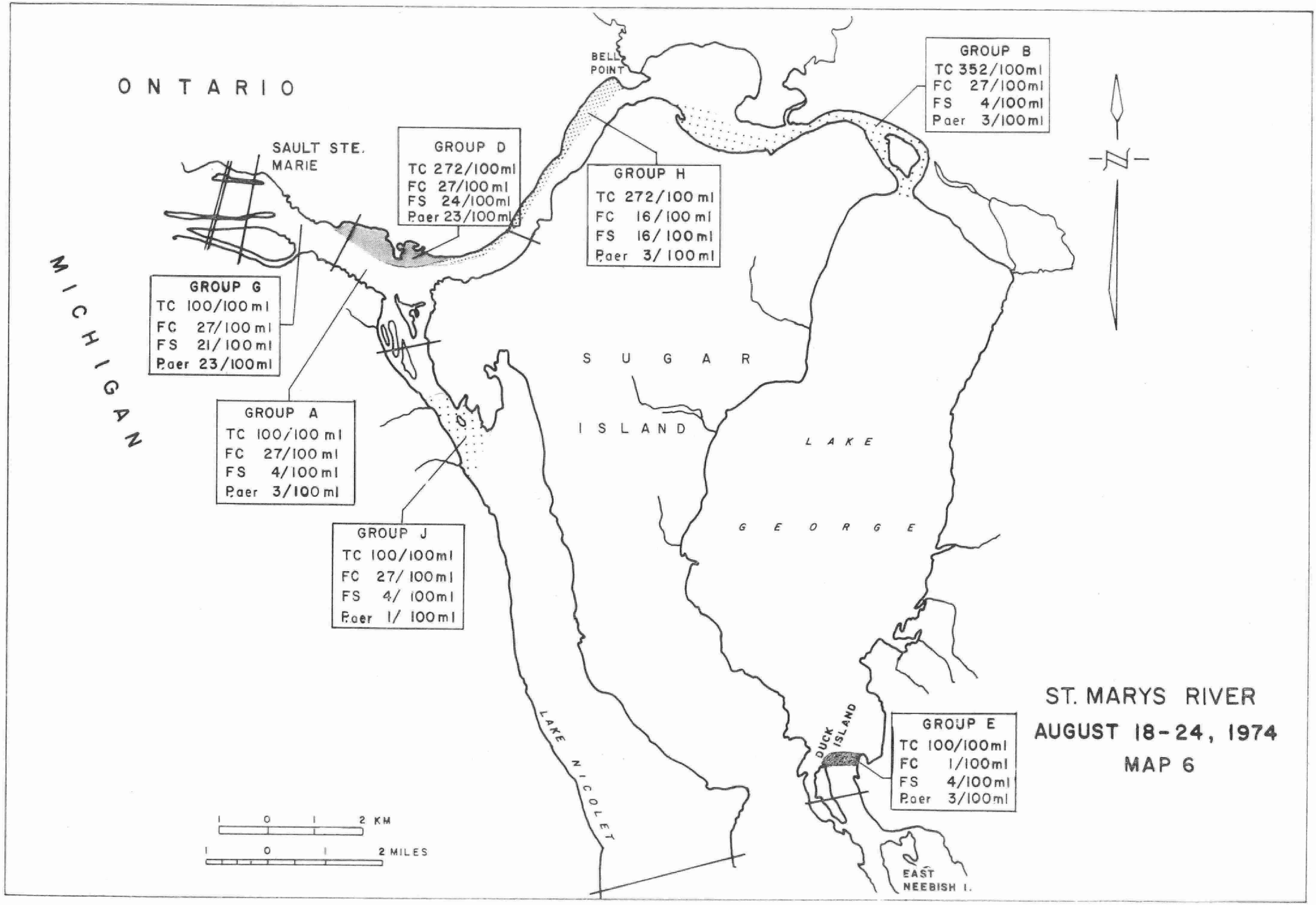
During the June survey (Map 5), the American side once again, was less impaired than the Canadian side. Stations closer to the American side had 15 TC, 3 FC and 3 FS/100 ml (Grp.A), while the Canadian side just below the locks up to Hog Island had 153 TC, 3FC, and 14 FS/100 ml (Grp.B). Two miles downstream from the locks (Stn 10 (4200'), very high densities of 2960 TC, 364 FC and 386 FS/100 ml occurred, reflecting the storm sewer input and a very high degree of impairment. Lower bacterial levels downstream of the STP (Grp.C) might be due to increased chlorination by the STP at that time to meet heavier demands posed by Domtar and Algoma Steel.

All stations except those close to the Government dock (10 (4200) had a Pseudomonad concentration of 2/100 ml. The level at this outfall station of 93 P.aer/100 ml further confirmed marked water quality deterioration around this area and the presence of recent fecal inputs.

During the August survey (Map 6), the area just downstream of Whitefish Island to the Government docks on the Canadian side (Ranges 29 to 10) had 272 TC, 27 FC and 24 FS/100 ml (Grp. D).


ONTARIO
MICHIGAN
SAULT STE. MARIE
BELL POINT
FRECHETTE POINT
GROUP B
TC 72/100ml
FC 17/100ml
FS 2/100ml
GROUP E
TC 10/100ml
FC 19/100ml
FS 2/100ml
GROUP D
TC 10/100 ml
FC 17/100 ml
FS 2/100 ml
GROUP A
TC 10/100ml
FC 2/100 ml
FS 2/100ml
S U G A R
I S L A N D
L A K E
G E O R G E
LAKE N. COLET
N
1 0 1 2 KM
1 0 1 2 MILES
ST. MARYS RIVER
APRIL 26-29, 1974
MAP 4


ONTARIO
MICHIGAN
BELL POINT
SAULT STE. MARIE
GROUP C
TC 71.5/100ml
FC 3/100 ml
FS 3/100ml
GROUP B
TC 153/100ml
FC 3/100ml
FS 14/100 ml
GROUP A
TC 15/100ml
FC 3/100ml
FS 3/100ml
Paer 2/100ml
STATION 10(4200)
TC 2960/100 ml
FC 364/100 ml
FS 386/100 ml
P.aer 93/100 ml
FRECHETTE POINT
SUGAR ISLAND
LAKE GEORGE
LAKE N'COLET
N
0 1 2 KM
0 1 2 MILES
ST. MARYS RIVER
JUNE 16-20, 1974
MAP 5


ONTARIO
MICHIGAN
SAULT STE. MARIE
BELL POINT
SUGAR ISLAND
LAKE GEORGE
LAKE NICOLET
DUCK ISLAND
EAST NEEBISH I.
GROUP B
TC 352/100ml
FC 27/100ml
FS 4/100ml
Paer 3/100ml
GROUP D
TC 272/100ml
FC 27/100ml
FS 24/100ml
Paer 23/100ml
GROUP H
TC 272/100 ml
FC 16/100 ml
FS 16/100 ml
Paer 3/100 ml
GROUP G
TC 100/100 ml
FC 27/100 ml
FS 21/100 ml
Paer 23/100ml
GROUP A
TC 100/100 ml
FC 27/100 ml
FS 4/100 ml
Paer 3/100 ml
GROUP J
TC 100/100ml
FC 27/100ml
FS 4/100ml
Paer 1/100ml
GROUP E
TC 100/100ml
FC 1/100ml
FS 4/100ml
Paer 3/100ml
N
1 0 1 2 KM
1 0 1 2 MILES
ST. MARYS RIVER
AUGUST 18-24, 1974
MAP 6

7C

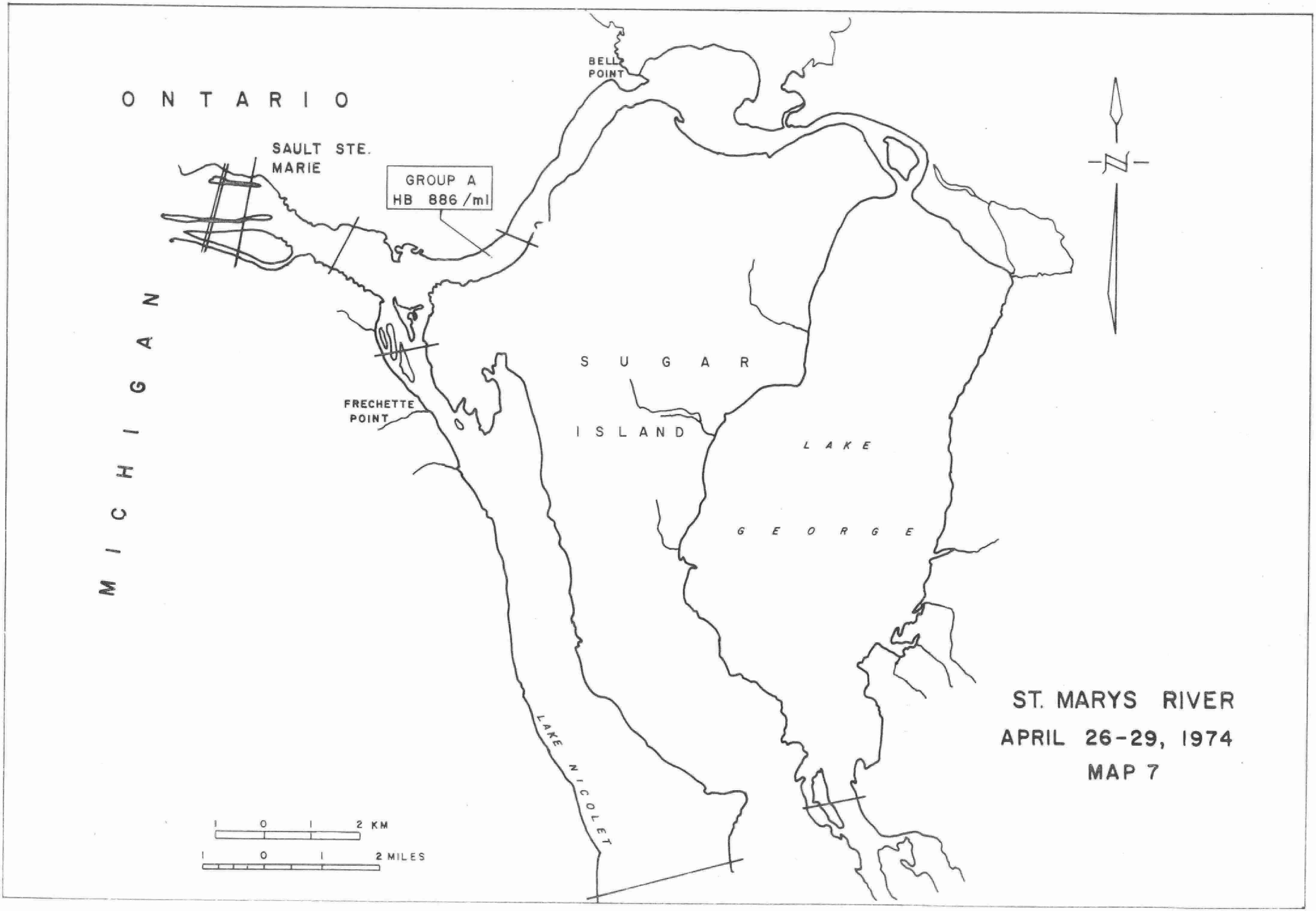
The area about Palmers Point and around Squirrel Island had a higher TC level of 352/100 ml (Grp.B). The American side and part of the channel leading to Little Lake George had 100 TC, 27 FC and 4 FS/100 ml (Grp.A). The improved water quality from the June survey on the Canadian side downstream from the STP and deterioration around Squirrel Island during the August survey might be attributed to currents causing transboundary movement of contaminants from the northern to southern areas of the channel.

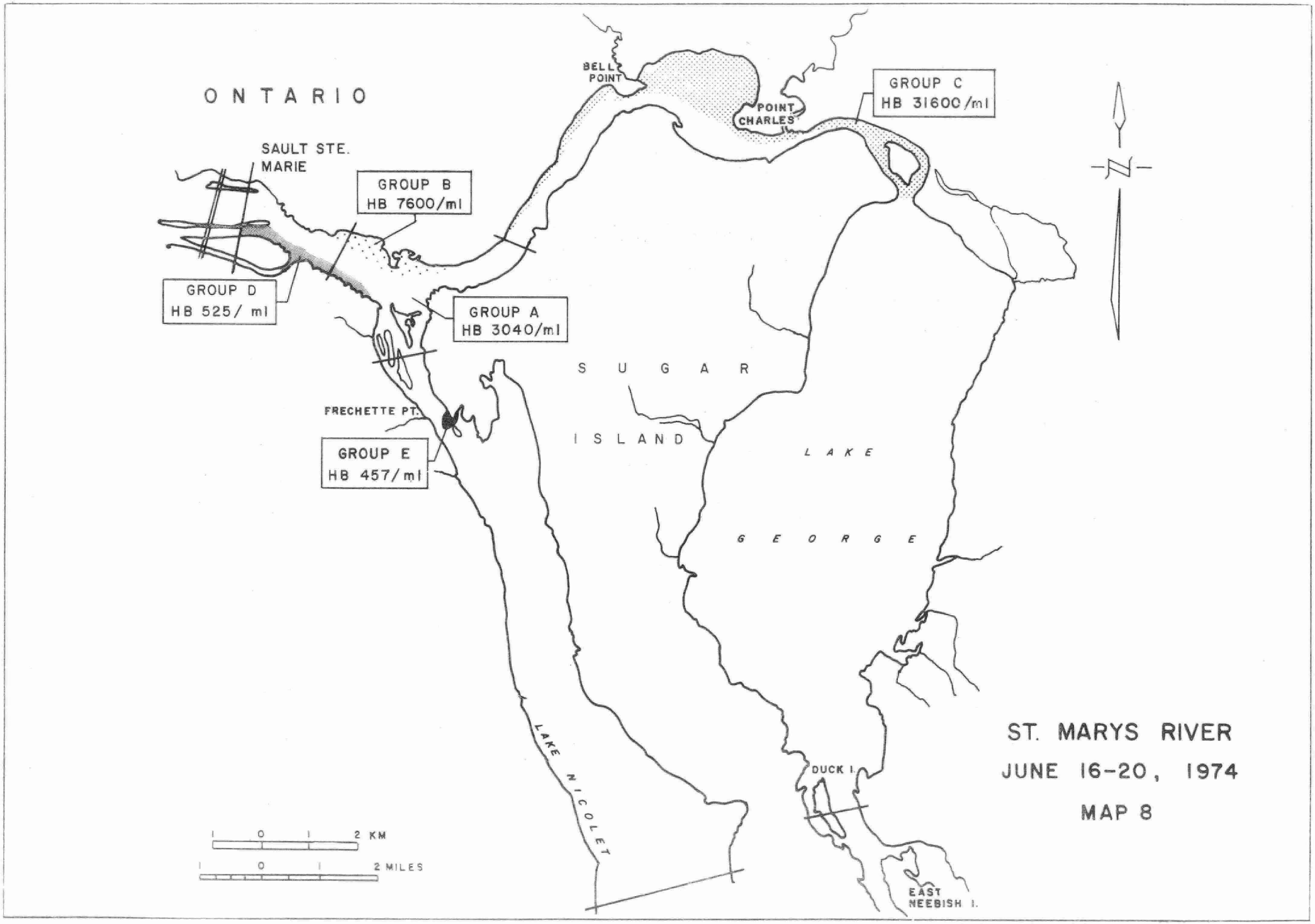
The majority of the survey area had P.aer. levels of 3/100 ml (Grp.A) while the area downstream from the locks and to the Government docks on the Canadian side (Range 10) had higher P.aer. levels of 23/100 ml, once again indicating recent fecal pollution.

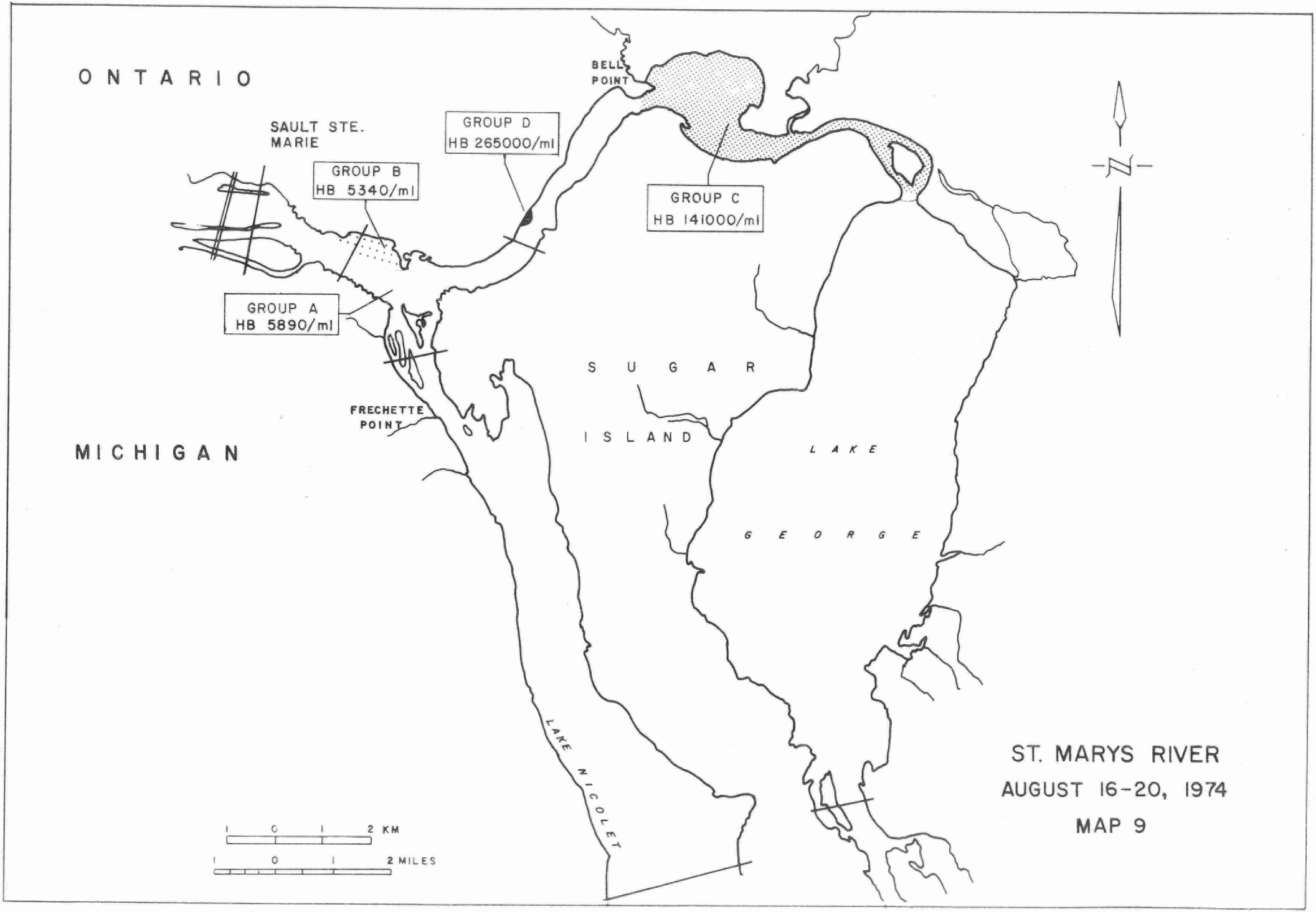
In April (Map 7) the HB population was homogeneous throughout the river at 886/ml (Grp.A).

In June (Map 8), the northern section of the Channel from the vicinity of the STP to Lake George had a HB concentration of 31600/ml (Grp.C), suggesting higher nutrient levels in this area while the upstream area had significantly lower levels

In August (Map 9) just downstream of the Sewage Treatment Plant, the HB population was 265000/ml but decreased to 141000/ml around Little Lake George and Squirrel Island. The remaining areas had an HB population of 5890/ml.


ONTARIO
MICHIGAN
BELL POINT
SAULT STE. MARIE
GROUP A
HB 886/ml
FRECHETTE POINT
SUGAR ISLAND
LAKE GEORGE
LAKE NICOLET
N
1 0 1 2 KM
1 0 1 2 MILES
ST. MARYS RIVER
APRIL 26-29, 1974
MAP 7


ONTARIO
SAULT STE. MARIE
GROUP B
HB 7600/ml
GROUP D
HB 525/ ml
GROUP A
HB 3040/ml
GROUP E
HB 457/ml
GROUP C
HB 31600/ml
BELL POINT
POINT CHARLES
FRECHETTE PT.
S U G A R
I S L A N D
L A K E
G E O R G E
LAKE NICOLET
DUCK I.
EAST NEEBISH I.
N
1 0 1 2 KM
1 0 1 2 MILES
ST. MARYS RIVER
JUNE 16-20, 1974
MAP 8


ONTARIO
MICHIGAN
SAULT STE. MARIE
BELL POINT
GROUP D
HB 265000/ml
GROUP B
HB 5340/ml
GROUP C
HB 141000/ml
GROUP A
HB 5890/ml
FRECHETTE POINT
S U G A R
I S L A N D
L A K E
G E O R G E
LAKE NICOLET
1 0 1 2 KM
1 0 1 2 MILES
ST. MARYS RIVER
AUGUST 16-20, 1974
MAP 9

Summary 1974

During the surveys, the Canadian shoreline displayed considerable impairment compared to the American shoreline.

The majority of stations (Grp.A) had successively higher bacterial concentrations with each survey. FC and FS concentrations were similar for the April and June surveys but the August survey revealed FC higher than FS densites for most stations (Grp.A).

Along the Canadian shoreline from Whitefish Island to the Government docks (Ranges 29 to 10), FC to FS densities decreased from April to June but increased again in the August survey. Levels at the storm sewer input (Stn 10 (4200')) were consistenly very high.

The Canadian shoreline downstream from the Sewage Treatment Plant had TC levels that exceeded the MOE Recrational Criteria in June. However the TC concentrations in August became concentrated towards the southern portion of the channel and around Squirrel Island possibly as a result of current changes.

HB populations were low throughout the entire river in April.

The June survey had a higher HB population than the August survey around the Government dock area on the Canadian side.

In August the area from the STP to Churchville Point (Range 18) had higher HB populations than in April or June with a very high HB population concentrated around the STP in August (Grp.D).

Comparison of 1973 and 1974 St. Mary's River Surveys

In 1974 all samples were taken downstream from the locks and included ranges 18 and 21 while omitting upstream ranges 2 and 33 sampled in 1973. Thus, a comparison will only be made for those stations that were sampled both years.

In the spring of 1973 bacterial densities formed a homogeneous area along the Canadian shoreline. In 1974, there was a TC and FC increase over 1973 along the Canadian shoreline with especially higher TC densities near the STP. FS concentrations decreased from 1973 to 1974 along the Canadian shoreline.

In the summer, TC densities increased from 1973 to 1974 below the STP while FC and FS remained about the same. High bacterial densities in 1974 was mainly limited to Canadian waters but in 1973 contamination spread towards the American shoreline and to Lake Nicolet. The majority of stations (Grp.A) had lower bacterial levels in 1974 than in 1973.

The fall 1973 bacterial densities were higher along the Canadian shoreline than in 1974. The majority of stations (Grp.A) had similar TC and FS densities both years but the FC concentration was greater in 1974 than in 1973.

Though bacterial levels during both years were generally below I.J.C. objectives and MOE criteria it should be noted the levels are kept from building to very high concentrations by the continuous flushing of the river with clean water from Lake Superior. Even with the diluting effect of the river, MOE criteria are sometimes exceeded, and the presence of P.aer. indicates that a health hazard could exist for people using the water. It would thus appear that abatement measures should be taken to reduce bacterial input into the river.

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A BACTERIOLOGICAL SURVEY OF THE SERPENT RIVER - 1975

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MINISTRY OF THE ENVIRONMENT

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BACTERIOLOGICAL SURVEY OF THE SERPENT RIVER - 1975

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ABSTRACT

An interim survey was carried out at the mouth of the Serpent River in May 1975, in order to assess the bacteriological impact on Serpent Harbour of the mining activities upstream. Very low levels of sanitary indicator bacteria were detected, and P. aeruginosa was never isolated. An iodimetric-titration procedure was used in the determination of sulphur-oxidizing bacteria. These bacteria were present in much larger concentrations than either the sanitary indicator or sulphate-reducing bacteria.

BACTERIOLOGICAL SURVEY OF THE SERPENT RIVER - 1975

INTRODUCTION

In accordance with the requirements of the Upper Great Lakes Reference Studies to define localized areas of impact, a survey was carried out in May, 1975, to assess the water quality at the mouth of the Serpent River. This report covers the bacteriological aspects of the water quality.

The Serpent River drains an area where substantial uranium mining takes place, and flows into the North Channel of Lake Huron. The drainage basin of the Serpent River is not heavily populated and the mining industry is the only major industry in the basin. Most of the mining takes place 30 miles from the mouth of the river.

METHODS

Field Procedures:

Bacteriological samples were collected at 19 stations located at the mouth of the Serpent River. Samples were collected daily from May 27th to May 29th, 1975. Each station was sampled twice daily, once in the morning and once in the afternoon.

All samples were taken in duplicate, and were collected approximately 1.5 metres below the water's surface, in sterile 6 oz. glass bottles. The duplicate sample was retained for MPN analyses. All samples were stored on ice and transported to the mobile laboratory in Sault Ste. Marie, where they were refrigerated until analyzed.

Lab Procedures:

All samples were analyzed for total coliform (TC), fecal coliform (FC), fecal streptococcus (FS) and Pseudomonas aeruginosa. Analyses for these parameters were performed within twelve hours of sampling and counts were recorded as numbers of organisms per 100 ml.

...2

Membrane filtration methods were performed according to Standard Methods (13th edition), using M-endo Agar LES (Difco) for TC, MacConkey Membrane Broth (Oxoid) for FC, M-enterococcus Agar (Difco) for FC and mPA Agar (Levin & Cabelli, 1972) for P. aeruginosa.

In addition, a determination of the heterotrophic bacterial poulation was performed within twelve hours of sampling. Analysis was done on a modified Foot and Taylor Agar (Appendix 1) using the spot plate technique. Incubation was for 7 days at 20°C and counts were recorded as number of organisms per 1 ml.

A most probable number (MPN) method was employed for the enumeration of sulphate-reducing bacteria (e.g. Desulfovibrio sp.) and chemoautotrophic sulphur-oxidizing bacteria (Thiobacillus sp.). A three-tube, three dilution series was employed. All MPN analyses were carried out within 5 days of sampling.

All tubes, including sterile controls, were incubated in the dark at 20°C for four weeks. The tubes were screw-capped and were not shaken during the incubation period. After incubation, all tubes were read, and the MPN of bacteria per 100 ml was determined using a standard MPN table.

Sulphate reducers were analyzed using API with tryptone (see Appendix 1). Two hours prior to inoculation, the tubed media was boiled to drive out the dissolved oxygen. One percent ascorbic acid, 1% ferrous ammonium sulphate, and a nail, was added to each tube just prior to inoculation. After inoculation, each tube was layered with 1-2 cm of sterile paraffin oil to ensure anaerobic incubation. After incubation, all tubes with a black precipitate of ferrous sulfide were considered positive.

Sulphur oxidizers were enumerated using Postgate's (1966) formulation (Appendix 1). Positive tubes were determined using an iodimetric-starch titration of the thiosulphate remaining in each tube (Appendix 11).

...3

Statistical Methods:

The geometric mean and variance were calculated for each parameter at each station. An analysis of variance (ANOVA) calculation was used to group the stations into statistical regions. The ANOVA analysis was first performed on all survey stations with all data being logarithmically transformed. In addition, the homogeneity of the variances was also checked using Bartlett's X^2 test of homogeneity. Where the calculated F-ratio and X^2 value were less than the critical value (0.05 level), the stations were considered statistically the same and were summarized as a group with one set of overall group statistics. If either the F or X^2 values were significant, then stations were withdrawn until both were non-significant. The statistical analyses were then repeated on the withdrawn stations until all stations had been properly grouped.

Criteria:

When full treatment is supplied, the criteria considered permissable for public surface water supplies for total coliform, fecal coliform, fecal streptococcus and heterotrophic bacterial counts, are maximum geometric means of 5,000, 500, 50 and 100,000 per 100 ml respectively. The maximum permissable levels for private water supplies requiring chlorination only are 100, 10, 1 and 1000 per 100 ml respectively, while those for waters requiring chlorination and filtration are 400, 40, 4 and 4,000 per 100 ml respectively.

The Recreational Use criteria states that: "Where ingestion is probable, recreational waters can be considered impaired when the coliform, fecal coliform, and/or enterococcus geometric mean density exceeds 1,000, 100 and/or 20 per 100 ml respectively . . . ". The main use of FS is not so much in its actual concentration, but rather in a ratio to fecal coliforms. "Where this ratio (FC/FS calculated from geometric means) exceeds 4.0, the source of bacterial contamination is likely to be human in origin. A ratio of less than 0.7 indicates an animal or

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storm water source of bacterial contamination" (Geldreich, 1969). It should be noted that this ratio can only be used to determine the source of pollution and not the safety of the water as animals are potential sources of organisms pathogenic to humans. In addition, it can only be applied at, or very close to, the source of pollution as the ratio may change rapidly with time.

RESULTS AND DISCUSSION:

The results are presented in Table 1 and illustrated in Fig. 1 and Fig. 2.

Very low levels of sanitary indicator bacteria were found at the mouth of the Serpent River. P. aeruginosa was not isolated from any sample analyzed, and there was no evidence of any serious fecal contamination in the Serpent Harbour region. The heterotrophic bacterial levels were also low.

The sulphate-reducing bacteria were only found at low levels. Their numbers were highest at the mouth of the river and decreased steadily throughout the outer harbour area. An elevated concentration of sulphate-reducing bacteria was evident near the outflow of a drainage ditch near the town of Spragge. This is a marshy region and probably more anoxic than the other sampling locations.

The sulphur-oxidizing bacterial concentrations are lowest at the mouth of the river, and their concentrations increase toward the open waters of the North Channel. The waters of the North Channel in this region are relatively high in sulphur content due to the sulphate input from the mining activities on the Serpent River, and probably due to some input from the natural strata of the region. In addition, fallout or washout from the air of sulphur compounds generated by the mining industry smoke stacks in Sudbury could contribute substantially.

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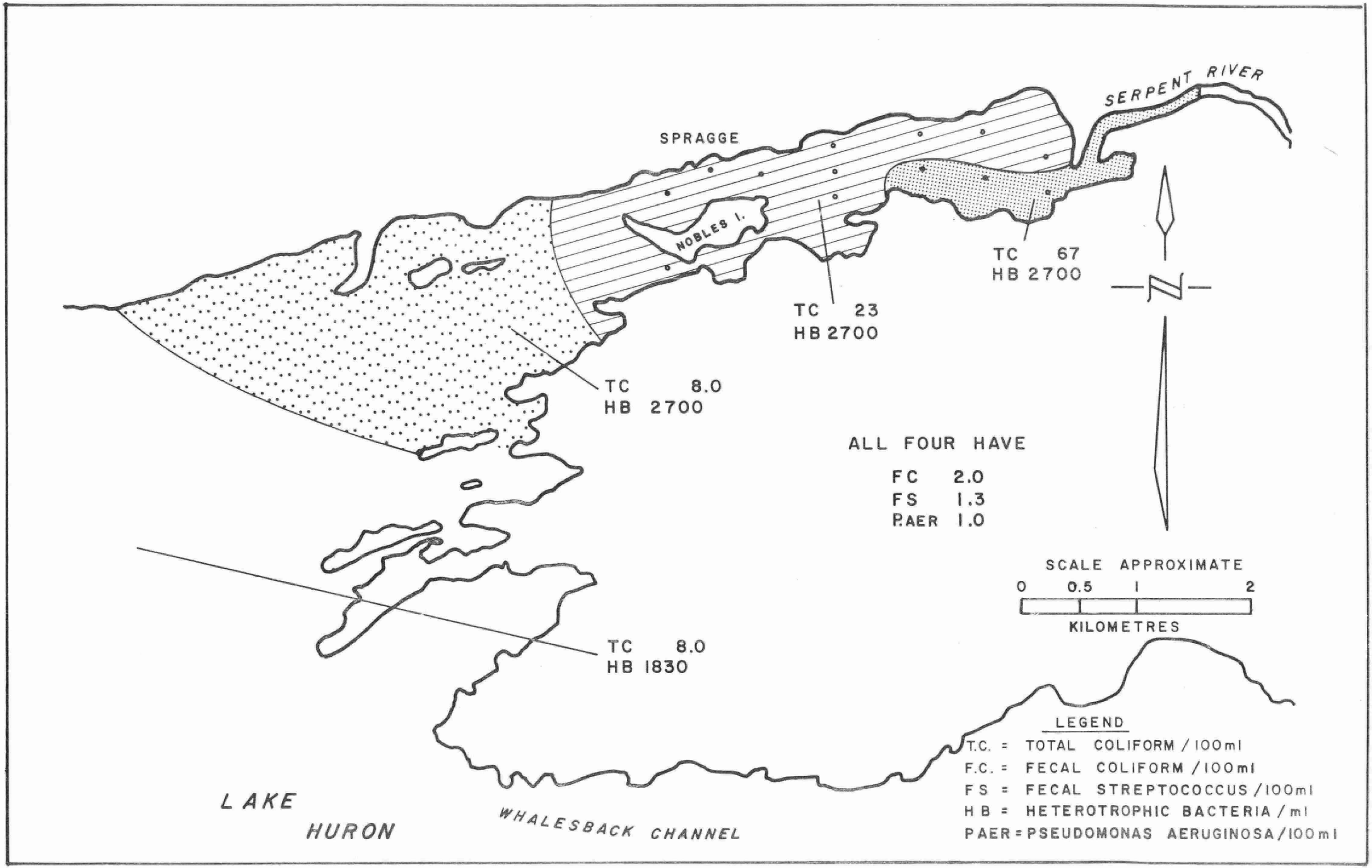

FIG I SERPENT RIVER BACTERIOLOGICAL SURVEY- MAY 1975.

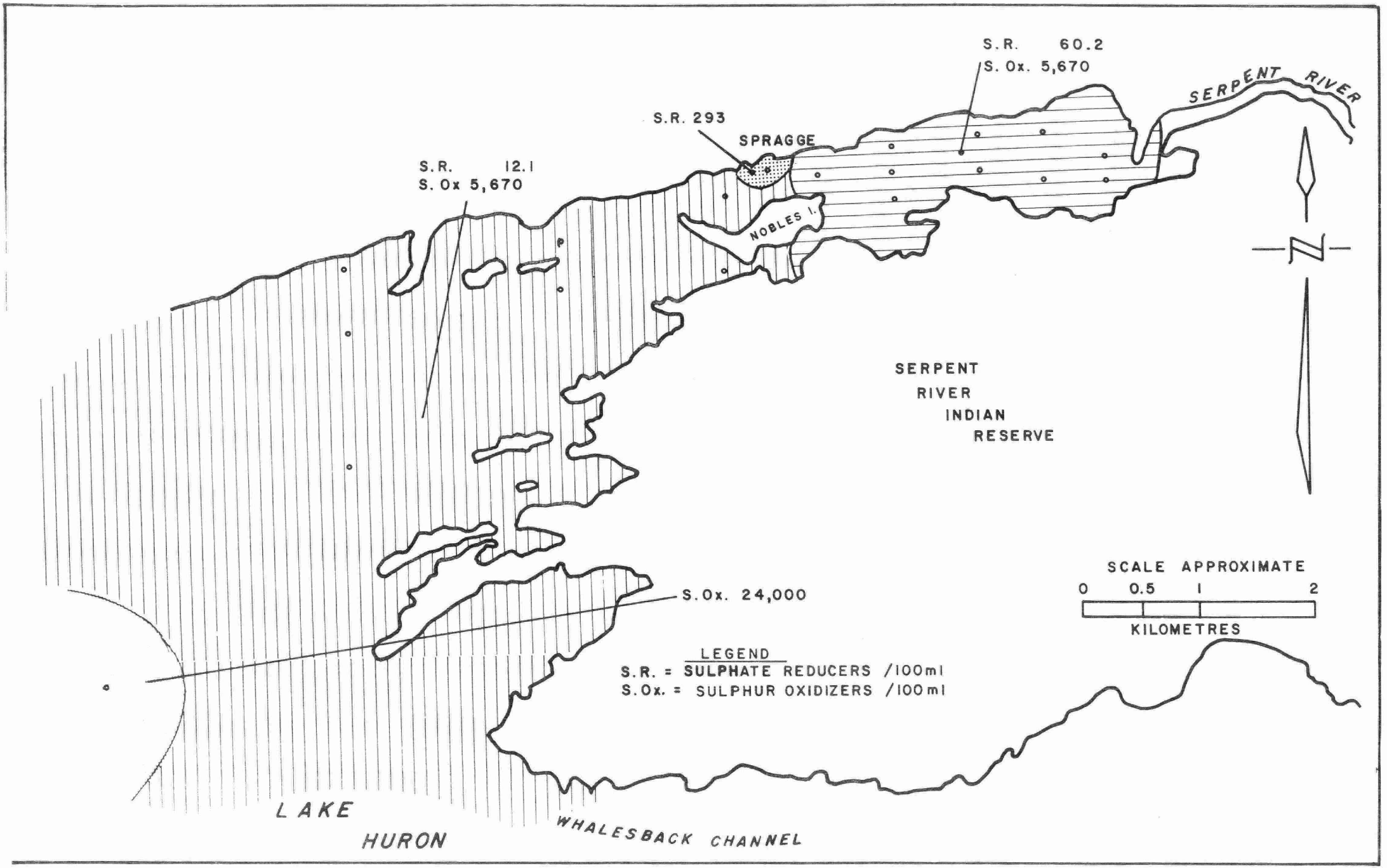

FIG 2 SERPENT RIVER – MAY 1975 – SULPHUR CYCLE BACTERIA.

TABLE 1: SERPENT RIVER - MAY 1975

PARAMETER	Group	N	S^2Log GM	GM	F	F (.05)	X^2	df
Total Coliforms	all*	119	0.3339	21.5	3.46	1.73	27.39	18
	A	68	0.2653	23.3	1.41	2.07	13.58	9
	B	30	0.3237	8.0	0.64	2.64	3.51	5
	C	21	0.0775	67.0	0.90	3.58	1.03	2
Fecal Coliforms	all*	118	0.1321	2.0	0.78	1.73	7.71	18
Fecal Streptococci	all*	119	0.0617	1.3	1.63	1.73	invalid	
Heterotrophic Bacteria	all*	111	0.0676	2,619.0	1.33	1.74	45.19	18
	A	102	0.0466	2,702.0	1.41	1.79	24.48	15
	B	9	0.3122	1,834.0	1.01	5.60	0.86	1
Pseudomonas Aeruginosa	all*	119	0.0000	1.0	-	-	-	-
Sulphur Oxidizers	all*	69	0.3083	5,913.2	1.52	1.83	33.69	18
	A	67	0.3061	5,671.0	1.41	1.86	15.74	17
	B	2	0.0000	24,000.0	-	-	-	-
Sulphate Reducers	all*	81	0.4006	39.1	4.08	1.79	19.52	18
	A	50	0.2626	60.2	1.95	2.15	8.31	9
	B	26	0.1923	12.1	1.49	2.60	7.35	7
	Stn. 293	5	0.6714	243.0	-	-	-	-

* all = all samples analyzed

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CONCLUSIONS

The waters of the Serpent Harbour region are very clean and no evidence of serious fecal contamination was evident.

The greatly elevated sulphur-oxidizing bacterial levels in the North Channel reflect the high sulphur content and well-oxygenated conditions of the water.

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APPENDIX 1

A. Foot and Taylor Agar (modified) - for heterotrophic bacteria

Peptone	0.5 g
K_2HPO_4	0.2 g
$MgSO_2$	0.05 g
$FeCl_3$	Trace
Soluble Casein	0.5 g
Agar	20 g
d H_2O	1000 ml

pH 7.2 Autoclave 15 min/121°C

B. API with tryptone (Thompson et al, 1976) - for sulfate-reducers

Yeast Extract	1.0 g
Tryptone	3.0 g
Sodium Lactate (60%)	8.6 g
Magnesium Sulphate Septahydrate	0.2 g
Dipotassium Hydrogen Phosphate	0.01 g
* Ascorbic Acid	0.1 g
* Ferrous Ammonium Sulphate	0.1 g
d H_2O	1000 ml

* The ascorbic acid and ferrous ammonium sulphate are prepared separately, each as 1% solutions, filter sterilized, and added to the medium just prior to the inoculation.

pH 7.5 Autoclave 10 min/121°C

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Thioparus Broth	(Postgate, J.R., 1966) - for sulphur oxidizers
Sodium Thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$)	5.0 g
Ammonium Chloride (NH_4Cl)	1.0 g
Potassium Phosphate (KH_2PO_4)	3.0 g
Calcium Chloride ($CaCl_2.2H_2O$)	0.33 g
Magnesium Sulphate ($Mg\ SO_4 \cdot 7H_2O$)	1.0 g
Trace Element Solution	1.0 ml
dH_2O	1000 ml

Adjust pH to 7.15

Autoclave 10 min/121°C — Final pH 7.2

Trace Element Solution

Solution 1	Ferrous Sulphate	19.50 g
	Zinc Sulphate	10.80 g
	Manganese Chloride	9.20 g
	dH_2O	250 ml
Solution 2	Ammonium Molybdate	2.60 g
	Cupric Sulphate	1.00 g
	Cobaltous Chloride	0.60 g
	dH_2O	250 ml
Solution 3	EDTA (Disodium Magnesium Salt)	65.86 g
	dH_2O	250 ml
Solution 4	Boric Acid	0.57 g
	dH_2O	250 ml

Add solution to total volume 1000 ml

Adjust pH to 6.0 with 0.5N KOH, filter sterilize

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APPENDIX 11

The utilization of sodium thiosulphate ($Na_2S_2O_3$) in Postgate's (1966) medium is a positive indication of the presence of active sulphur-oxidizing bacteria (Thiobacillus sp.). The oxidation of $Na_2S_2O_3$ can be assessed by an iodimetric titration procedure utilizing a starch indicator.

Method:

A. Preparation of 0.1N Iodine solution

1) Add 20.0 g of potassium iodine (acetate-free) to 30-40 ml of distilled H_2O in a glass-stoppered one litre flask.

2) Weigh out 12.69 g iodine crystals* on a rough balance and add to the potassium iodide solution.

* Note: Be very careful when handling iodine, it is a very powerful oxidant.

3) Shake solution until all iodine has dissolved, then allow flask to attain ambient temperature.

4) Add distilled H_2O to bring volume to 1000 ml.

5) Cover flask with aluminum foil and store in dark at $4^{\circ}C$.

B. Preparation of Starch Indicator Solution:

1) Make a paste of 1 g soluble starch and 5 ml of distilled H_2O.

2) Add the paste, with constant stirring, to 100 ml boiling water and boil solution for 1 minute.

3) Allow solution to cool to ambient temperature, then add 3 g potassium iodine (acetate-free).

The starch solution may be stored at $4^{\circ}C$ for up to 48 hours.

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C. Preparation of Starch Indicator Solution:

M.W. $Na_2S_2O_3 . 5H_2O$ = 248.20

1) Weigh out 24.82 g $Na_2S_2O_3.5H_2O$ (pure) into 1000 ml of previously boiled water that has cooled to ambient temperature.

2) Add 1.0 ml chloroform ($CHCl_3$) and store at 4^oC in dark (maximum storage - 1 week).

D. Normalization of Iodine Solution:

The normality of the iodine solution is determined by titration against freshly prepared 0.1N sodium thiosulphate solution.

1) Transfer 25.0 ml iodine solution to a 250 ml conical flask.

2) Dilute to 100 ml with water.

3) Add 0.1N $Na_2S_2O_3$ solution until a pale yellow colour remains.

4) Add 2 ml starch solution.

5) Add thiosulphate solution slowly until solution is just colourless.

6) Repeat titration. Titrations should agree within 0.1 ml.

7) Determine normality of iodine solution

$$N_1 = \frac{N_2V_2}{V_1}$$

where N_1 = normality of iodine solution

N_2 = normality of thiosulphate solution

V_1 = volume of iodine solution

V_2 = volume of thiosulphate solution

$\therefore$ Normality of iodine solution = $\frac{0.1 \times V_2}{25.0}$

= 0.004 x volume of thiosulphate solution.

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E. Titration Procedure:

Determine the amount of $Na_2S_2O_3 \cdot 5H_2O$ in uninocated control tubes as follows:

1) Add 0.2 ml starch solution to each control tube.

2) Titrate the control tubes with the iodine solution.

The end point is indicated by the appearance of a pale blue colour throughout the tube.

To determine the 95% confidence limits of the titration, determine the mean, $\bar{x}$, and the standard deviation.

e.g. 95% confidence interval $= \bar{x} \pm ts$

where $\bar{x}$ = mean or average

s = standard deviation

$$= \pm \sqrt{\frac{(x-\bar{x})^2}{n-1}}$$

n = number of control tubes

t = compensating variable dependent on number of control tubes.

The amount of iodine to be added to each sample tube is equivalent to

$$\bar{x} - ts$$

Where the number of control tubes is ten,

t = 2.26, and the amount of iodine to be added

is $\bar{x} - 2.26\ s$.

Sample tubes that turn blue with the addition of the pre-determined amount of iodine are positive, all other tubes are negative.

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A BACTERIOLOGICAL SURVEY OF THE SPANISH RIVER - 1975

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MINISTRY OF THE ENVIRONMENT

JANUARY 1978

BACTERIOLOGICAL SURVEY OF THE SPANISH RIVER - 1975

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ABSTRACT

An intensive three-day survey was undertaken in May 1975 in order to assess the microbiological water quality at the mouth of the Spanish River. The river is a receiving body for both kraft mill and municipal waste discharges. The parameters studied by membrane filtration techniques were total coliforms, fecal coliforms, fecal streptococci, and Pseudomonas aeruginosa. A spot plate method was used to assess aerobic heterotrophic bacterial concentrations, and MPN methodologies were used to measure sulphate-reducing and sulphur-oxidizing bacteria. The procedure for sulphur- oxidizers involved an iodimetric-starch titration methodology.

Elevated levels of all bacterial parameters were detected at the mouth of the Spanish River, and P. aeruginosa was frequently isolated. The highest sulphur-oxidizer populations detected in the North Channel in 1975 were detected off the mouth of the Spanish River.

BACTERIOLOGICAL SURVEY OF THE SPANISH RIVER - 1975

INTRODUCTION

Earlier water quality studies of the Lower Spanish River (OWRC, 1966 and MOE, 1972) indicated that the entire lower Spanish River, from Espanola to Brennan Harbour, was affected by waste discharge from the Kraft Pulp Mill at Espanola.

In accordance with Upper Great Lakes Reference Studies to define localized areas of impact, a study was carried out in May, 1975, to assess the water quality at the mouth of the Spanish River. This report covers the bacteriological aspects of the water quality.

The Spanish River drains approximately 5,400 square miles of the southern half of the District of Sudbury. It flows through the town of Espanola and continues 32 miles in a westerly direction to the North Channel of Lake Huron. The river receives municipal waste discharges from the town of Espanola, and all the process wastewaters from the Eddy Forest Products Limited Kraft Mill. These inputs are located 31 miles upstream from the mouth of the river. Two small towns, Massey and Webbwood, are located on the river between Espanola and the mouth. The Aux Sables River flows from the north into the Spanish River near Massey.

METHODS

Field Procedures:

Bacteriological samples were collected at 19 stations located at the mouth of the Spanish River. Samples were collected on the 22nd, 23rd and 25th of May, 1975. Each station was sampled twice daily, once in the morning and once in the afternoon. All samples were taken in duplicate, and were collected approximately 1.5 metres below the water's surface in sterile 6 oz. glass bottles. The duplicate sample was retained for the MPN analyses.

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All samples were stored on ice and transported to the mobile laboratory located in Sault Ste. Marie, where they were refrigerated until analyzed.

Laboratory Procedures:

All samples were analyzed for total coliform (TC), fecal coliform (FC), fecal streptococcus (FS) and Pseudomonas aeruginosa. Analyses for these parameters were performed within twelve hours of sampling and counts were recorded as number of organisms per 100 ml. Membrane filtration methods were performed according to Standard Methods (13th edition), using M-endo Agar LES (Difco) for TC, MacConkey Membrane Broth (Oxoid) for FC, M-enterococcus Agar (Difco) for FS, and mPA Agar (Levin and Cabelli, 1972) for Ps. aeruginosa.

In addition, a determination of the heterotrophic bacterial population was performed within twelve hours of sampling. Analyses was done on a modified Foot and Taylor Agar (Appendix I) using the spot plate technique. Incubation was for 7 days at 20°C and counts were recorded as number of organisms per ml.

A most probable number (MPN) method was employed for the enumeration of sulphate-reducing bacteria and chemoautotrophic sulphur-oxidizing bacteria (Thiobacillus sp.).

A three tube, three dilution system was employed. All MPN analyses were carried out within 5 days of sampling. All tubes, including sterile controls, were incubated and were not shaken during the incubation period. After incubation, all tubes were read, and the MPN of bacteria per 100 ml was determined using a standard MPN table.

Sulphate reducers were analyzed using API with tryptone (see Appendix I). Two hours prior to inoculation, the tubed media was boiled to drive out the dissolved oxygen. The 1% ascorbic acid, 1% ferrous ammonium sulphate, and a nail, was added to each tube just prior to inoculation. After incubation, all tubes with a black precipitate of ferrous sulphide were considered positive.

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Sulphur oxidizers were enumerated using Postgate's (1966) formulation (see Appendix I). Positive tubes were determined using an iodimetric-starch titration of the thiosulphate remaining in each tube (Appendix II).

Statistical Methods:

The geometric mean and variance were calculated for each parameter at each station. An analysis of variance (ANOVA) calculation was used to group the stations into statistical regions. The ANOVA analyses was first performed on all survey stations with all data being logarithmically transformed. In addition, the homogeneity of the variance was also checked using Bartlett's X^2 test of homogeneity. Where the calculated F-ratio and X^2 value were less than the critical F-ratio (0.05 level), the stations were considered statistically the same and were summarized as a group with one set of overall group statistics. If either the F or X^2 values were significant, then stations were withdrawn until both were non-significant. The statistical analyses were then repeated on the withdrawn stations until all stations had been properly grouped.

Criteria:

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The Recreational Use criteria state that: "Where ingestion is probable, recreational waters can be considered impaired when the coliform, fecal coliform, and/or enterococcus geometric mean density exceeds 1,000, 100 and/or 20 per 100 ml respectively . . .". The main use of FS is not so much in its actual concentration, but rather in a ratio to fecal coliforms. "Where this ratio (FC/FS

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calculated from geometric means) exceeds 4.0, the source of bacterial contamination is likely to be human in origin. A ratio of less than 0.7 indicated an animal or storm water source of bacterial contamination" (Geldreich, 1969). It should be noted that this ratio can only be used to determine the type of source of pollution and not the safety of the water, as animals are potential sources of organisms that are pathogenic to humans. In addition, it can only be applied at, or very close to the source of pollution, as the ratio may change rapidly with time.

RESULTS AND DISCUSSION

The results are presented in Table 1 and illustrated in Figures 1 and 2.

Elevated concentrations of sanitary indicator bacteria, significantly higher than concentrations normally evident in the North Channel (Young et. al.), are found at the mouth of the Spanish River. These bacterial concentrations approach, but do not exceed MOE criteria, for recreational waters. However, several factors indicate that there is a serious pollution problem at the mouth of the Spanish River.

The high (6:1) FC/FS ratio at the mouth of the river is indicative of human fecal input upstream, probably from the Espanola region. The fact that the bacterial levels are still so high, at a point 30 miles from the source, indicates that the bacterial loadings at the source are extremely high. It is significant that these levels were observed in May, when the water temperature is very low and peak bacterial levels have not been reached. It is also significant that Ps. aeruginosa was frequently isolated at the mouth of the Spanish river. This bacterium is rarely isolated from Great Lakes water unless there has been a recent input of fecal material (Jenkins et. al., 1977). Its presence at the mouth of the Spanish River indicates that a serious pollution problem exists upstream.

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The sulphate-reducing bacterial populations were highest at the mouth of the Spanish river where a great deal of sulphur compounds that have been carried downstream from the pulp and paper mills are present. The water at the mouth is more anoxic than the water further out in the North channel, and the sulphate-reducing bacterial population fell off rapidly. The high levels of sulphate-reducing bacteria (> 1,400/100 ml) are indicative of impaired water quality.

The sulphur-oxidizing bacterial population were highest in the region where the anoxic Spanish River water mixed with the more oxygenated water of the North Channel. These sulphur-oxidizer concentrations (> 45,000/100 ml) were the highest sulphur-oxidizer concentrations observed in the North Channel in 1975.

CONCLUSION

The mouth of the Spanish River is one of the most severely impaired regions, microbiologically, in the North Channel of Lake Huron. The magnitude of the problem at the mouth of the river, 30 miles from the sources, suggests that much more severe problems exist upstream, closer to the source.

Further studies need to be carried out to determine the extent of the problem during the summer months when the bacterial levels would probably be much higher and possibly in excess of the MOE criteria. Future surveys should also be designed to collect some samples nearer to Espanola and therefore closer to the source of the pollution.

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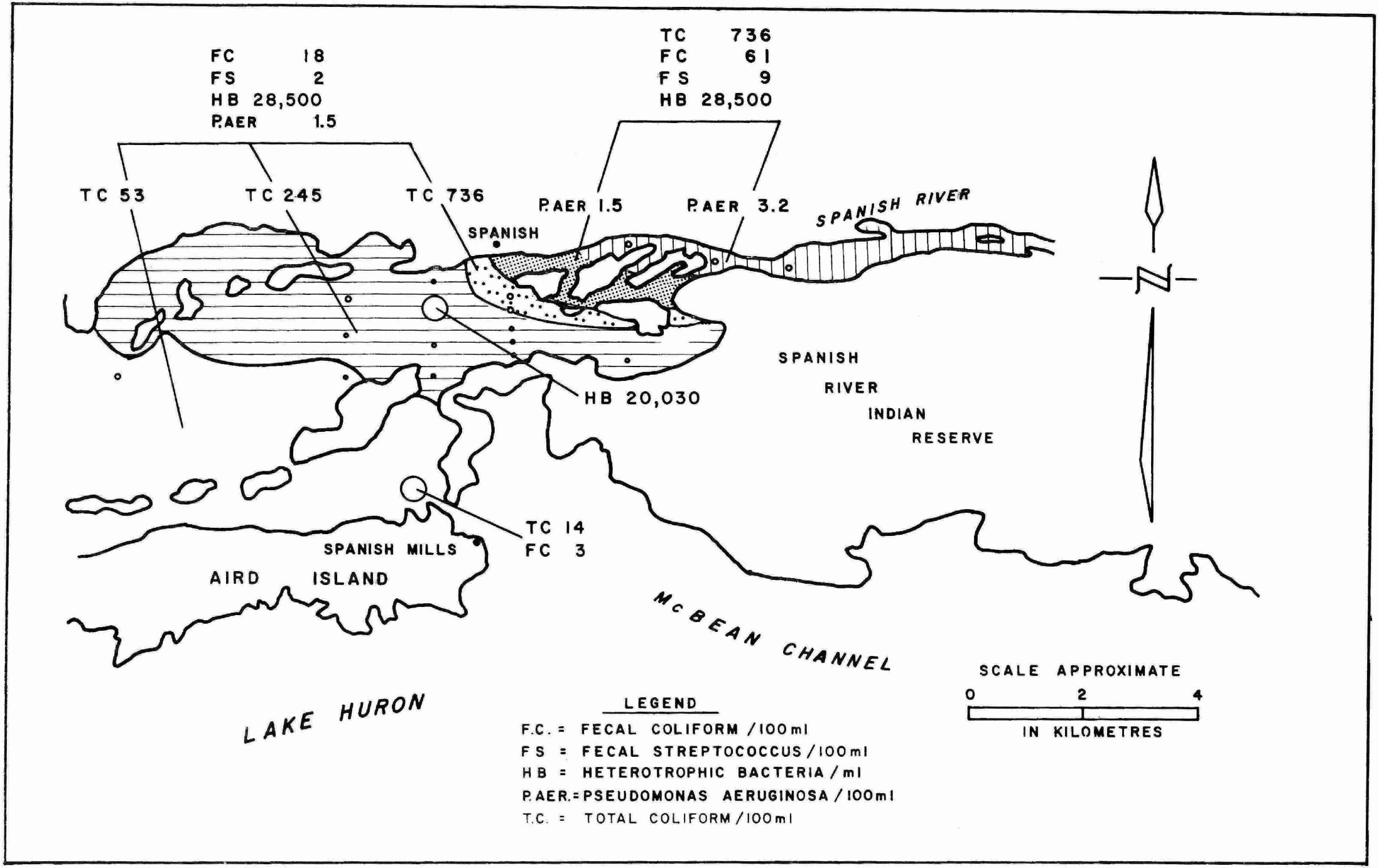

FIG I SPANISH RIVER BACTERIOLOGICAL SURVEY - MAY 1975.

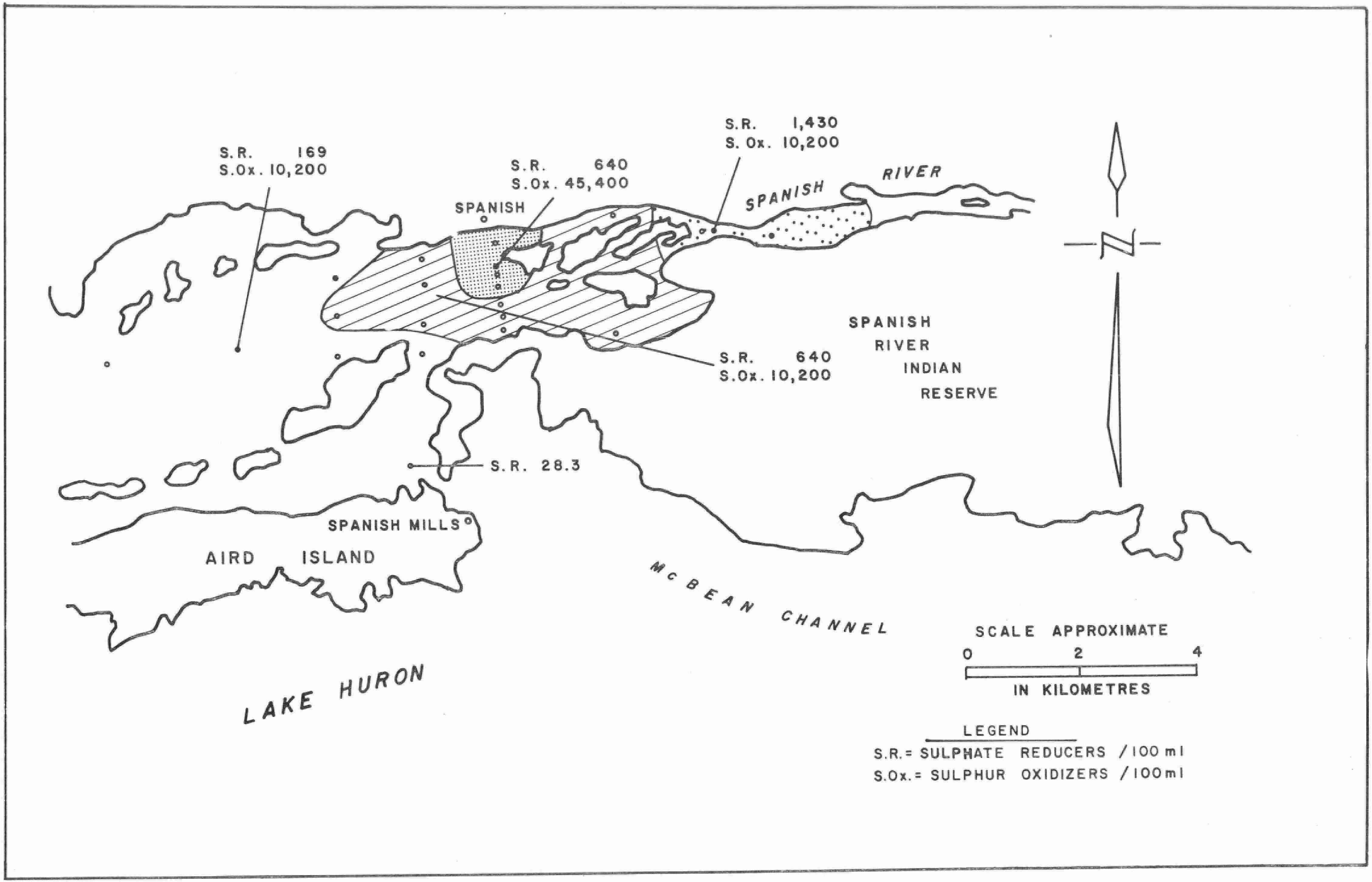

FIG 2 SPANISH RIVER - MAY 1975 - SULPHUR CYCLE BACTERIA.

TABLE I: - SPANISH RIVER SURVEY - MAY 1975

PARAMETER	GROUP	N	S^2	GM	F	F (.05)	X^2	df
Total Coliforms	A (All)	114	0.3225	254.7	10.91	1.74	20.40	18
	B	60	0.1243	245.2	1.99	2.10	11.79	9
	C	36	0.0921	736.3	0.28	2.55	4.16	5
	D	12	0.1735	53.4	0.01	4.97	0.03	1
	E	6	0.2983	14.5				
Fecal Coliforms	A (All)	114	0.3441	20.7	3.70	1.74	15.26	18
	B	84	0.3017	17.6	1.62	1.89	10.95	13
	C	24	0.1351	60.2	0.43	3.13	1.03	3
	D	6	0.1359	2.8				
Fecal Streptococci	A (All)	114	0.2214	2.5	5.92	1.74	invalid	
	B	90	0.1436	1.8	1.80	1.85	invalid	
	C	24	0.1337	8.9	2.57	3.13	7.79	3
Heterotrophic Bacteria	A (All)	108	0.0552	27,900	0.86	1.74	29.71	18
	B	102	0.0412	28,450	1.07	1.77	12.20	17
	C	6	0.3219	20,030				
Pseudomonas aeruginosa	A (All)	114	0.1018	1.7	2.26	1.74	invalid	
	B	96	0.0852	1.5	1.65	1.82	invalid	
	C	18	0.1107	3.2	0.03	3.71	2.93	2
Sulphur Oxidizers	All	114	0.3631	12,906	2.07	1.74	9.95	18
	A	96	0.3140	10,193	0.96	1.82	7.76	15
	B	18	0.2835	45,428	1.14	3.71	2.06	2
Sulphate Reducers	All	114	0.3996	415.6	4.86	1.74	29.45	18
	A	72	0.2517	600.1	0.86	1.98	13.65	11
	B	24	0.3335	145.9	0.79	3.13	3.03	3
	C	12	0.0278	1,426.7	0.00	4.97	0.00	1
	Stn. 10	6	0.1796	28.3	-	-	-	-

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APPENDIX I

A. Foot and Taylor Agar (Modified) - For Heterotrophic Bacterial Count

Peptone	0.5 g
K_2HPO_4	0.2 g
$MgSO_2$	0.05 g
$FeCl_3$	Trace
Soluble Casein	0.5 g
Agar	20.0 g
dH_2O	1000 ml
pH 7.2	Autoclave 15 min/121°C

B. API with Tryptone (Thompson et al, 1976) - For Sulphate Reducers

Yeast Extract	1.0 g
Tryptone	3.0 g
Sodium Lactate (60%)	8.6 g
Magnesium Sulphate Septahydrate	0.2 g
Dipotassium Hydrogen Phosphate	0.01 g
* Ascorbic Acid	0.1 g
* Ferrous Ammonium Sulphate	0.1 g
dH_2O	1000 ml

* The ascorbic acid and ferrous ammonium sulphate are prepared separately, each as 1% solutions, filter sterilized, and added to the medium just prior to inoculation.

pH 7.5 Autoclave 10 min/121°C

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THIOPARUS BROTH

(Postgate, J.R., 1966) - For Sulphur Oxidizers

Sodium Thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$)	5.0 g
Ammonium Chloride (NH_4Cl)	1.0 g
Potassium Phosphate (KH_2PO_4)	3.0g
Calcium Chloride ($CaCl_2 \cdot 2H_2O$)	0.33 g
Magnesium Sulphate ($MgSO_4 \cdot 7H_2O$)	1.0 g
Trace Element Solution	1.0 ml
dH_2O	1000 ml

Adjust pH to 7.15

Autoclave 10 min/121°C — Final pH 7.2

Trace Element Solution:

Solution 1	Ferrous Sulphate	19.50 g
	Zinc sulphate	10.80 g
	Manganese Chloride	9.20 g
	dH_2O	250 ml
Solution 2	Ammonium Molybdate	2.60 g
	Cupric Sulphate	1.00 g
	Cobaltous Chloride	0.60 g
	dH_2O	250 ml
Solution 3	EDTA (Disodium Magnesium Salt)	65.86 g
	dH_2O	250 ml
Solution 4	Boric acid	0.57 g
	dH_2O	250 ml

Add Solutions to total Volume 1000 ml

Adjust pH to 6.0 with 0.5N KOH, Filter Sterilize.

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APPENDIX II

The utilization of sodium thiosulphate ($Na_2S_2O_3$) in Postgate's (1966) medium is a positive indication of the presence of active sulphur-oxidizing bacteria (Thiobacillus sp.) The oxdiation of $Na_2S_2O_3$ can be assessed by an iodimetric titration procedure utilizing a starch indicator.

Method

(A) Preparation of 0.1N Iodine solution:

1) Add 20.0 g potassium iodide (acetate-free) to 30-40 ml of distilled H_2O in a glass-stoppered 1 litre flask

2) Weigh out 12.69 g iodine crystals* on a rough balance and add to the potassium iodine solution.

* Note Be very careful when handling iodine, it is a very powerful oxidant.

3) Shake solution until all iodine has dissolved, then allow flask to attain ambient temperature.

4) Add distilled H_2O to bring volume to 1000 ml.

5) Cover flask with aluminum foil and store in dark at $4^{o}C$.

(B) Preparation of Starch Indicator Solution:

1) Make a paste of 1 g soluble starch and 5 ml of distilled H_2O.

2) Add the paste, with constant stirring, to 100 ml boiling water, and boil solution for 1 minute.

3) Allow solution to cool to ambient temperature, then add 3 g potassium iodide (acetate-free). The starch solution may be stored at $4^{o}C$ for up to 48 hours.

(C) Preparation of 0.1N Sodium Thiosulphate Solution:

M.W. $Na_2S_2O_3 \cdot 5H_2O = 248.20$

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1) Weigh out 24.82 g $Na_2S_2O_3 \cdot 5H_2O$ (pure) into 1000 ml of previously boiled water that has cooled to ambient temperature.
2) Add 1.0 ml chloroform ($CHCl_3$) and store at 4^oC in dark (maximum storage - 1 week).

(D) Normalization of Iodine Solution:

The normality of the iodine solution is determined by the titration against freshly prepared 0.1N sodium thiosulphate solution.

1) Transfer 25.0 ml iodine solution to a 250 ml conical flask
2) Dilute to 100 ml with water
3) Add 0.1N $Na_2S_2O_3$ solution until a pale yellow colour remains
4) Add 2 ml starch solution
5) Add thiosulphate solution slowly until solution is just colourless
6) Repeat titration. Titrations should agree within 0.1 ml
7) Determine normality of Iodine solution

$$\frac{N_1 = N_2V_2}{V_1}$$

N_1 = normality of iodine solution
N_2 = volume of iodine
N_2 = normality of thiosulphate solution
N_2 = volume of thiosulphate solution

$\therefore$ Normality of iodine solution $= \frac{0.1 \times V_2}{25.0}$

$= 0.004 \times$ volume of thiosulphate solution.

(E) Titration Procedure:

Determine the amount of $Na_2S_2O_3 \cdot 5H_2O$ in uninoculated control tubes as follows:

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1) Add 0.2 ml starch solution to each control tube.

2) Titrate the control tubes with the iodine solution. The end point is indicated by the appearance of a pale blue colour throughout the tube.

To determine the 95% confidence limits of the titration, determine the mean, $\bar{x}$, and the standard deviation,

e.g. 95% confidence interval = $\bar{x} \pm ts$

where $\bar{x}$ = mean or average

s = standard deviation

$$= \pm \sqrt{\frac{(x - \bar{x})^2}{n-1}}$$

n = number of control tubes

t = compensating variable dependent on number of control tubes.

The amount of iodine to be added to each sample tube is equivalent to $\bar{x} - ts$.

Where the number of control tubes is ten, t = 2.26, and the amount of iodine to be added is $\bar{x} - 2.26\ s$.

Sample tubes that turn blue with the addition of the predetermined amount of iodine are positive, all other tubes are negative.

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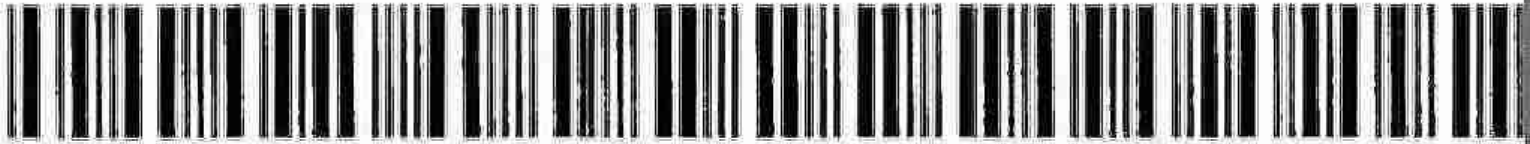
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